

Biodegradable composition with prolonged release of the biological active compound and preparation thereof

Field of invention

The invention relates to a biodegradable composition with prolonged release of a biological active compound and the preparation thereof.

Background of invention

High-molecular compounds based on polymers and copolymers of glycolic and lactic acids, possessing linear constitution of chains, have been used since the 1960's for production of surgical sutures and/or orthopedic joining devices [Middleton, J. C., Tipton, A.J., Synthetic biodegradable polymers as orthopedic devices, Biomaterials 21 (2000) 2335-2346]. DL-lactic acid/glycolic acid copolymers with higher ratios of lactic acid or with equimolar ratio of both monomers, have been extensively studied and commercially exploited for formulation of biodegradable implantable systems of particulate type [Arshady, R. (ed) : Microspheres, Microcapsules & Liposomes (1.ed.) vol. 2, pp. 1291, Citus, London 1999] or monolithic type [Rothen-Weinhold, A. et al., Injection-molding versus extrusion as manufacturing technique for the preparation of biodegradable implants, Eur. J. Pharm. Biopharm. 48 (1999) 113-121]. In most cases these implants or microparticles contain peptides with gonadotrophic activity. Information has been published about the advantageous release profiles of bromocriptin mesylate from the microparticulate systems based on the microspheres prepared from the high-molecular copolymers of glucose with lactic acid, synthesised by the ring-opening polymerisation [Kissel, T. et al., Parenteral depot-systems on the basis of biodegradable polyesters, J. Contr. Rel. 16(1991) 27-42].

A series of patents by Atrix Laboratories uses the principle of application of the solution of a biocompatible polymer as the minor component in a mixture with a biocompatible water-miscible solvent as

the markedly major component. After the application of the solution by syringe, the solvent is rapidly distributed into the adjacent muscle or other soft tissues and the *in situ* implant is formed [Dunn, R. L. et al., Biodegradable in-situ forming implants and methods of producing the same, US-Patent 4,938,763; Shively, M.L. et al., Physico-chemical characterization of a polymeric injectable implant delivery system, J. Contr. Rel. 33 (1995) 237-243; Kranz, H. et al., Myotoxicity studies of injectable biodegradable in-situ forming drug delivery systems, Int. J.Pharm.212(2001) 11-18; Coonts, B.A. et al., Biodegradation and biocompatibility of a guided tissue regeneration barrier membrane formed from a liquid polymer material, J.Biomed.Mater.Res.42(1998) 303-311]. This method of drug delivery has found a practical application in the pharmacotherapy (Yewey, G. et al., Liquid delivery compositions, PCTWO95/27481). A similar method for the *in situ* implant formation was carried out using glycofurole as in the aqueous medium miscible solvent of lactic acid/glycolic acid copolymer [Eliaz, R. E., Kost, J., Characterization of a polymeric PLGA-injectable implant delivery system for the controlled release of proteins, J. Biomed. Mater. Res. 50 (2000) 388-396]. Furthermore, a subcutaneous application of a gel by syringe with subsequent photopolymerisation of reagents applied and the cross-linking of the polymeric chains has been described [Elisseeff, J. et al., Transdermal photopolymerization for minimally invasive implantation, Proc. Natl. Acad. Sci. USA 96(1999)3104-3107]. Injectable protein compositions, based on the phase transitions of aqueous collagen solution at temperatures below 45 °C have been patented [Jones, R.E., Li, M.T., Novel collagen formulations, PCTWO96/33696].

Polyesters with branched chains have a higher random coil density compared to polyesters with a linear chain constitution. This is connected with their smaller volume and lower viscosity of their melts and solutions [Colby, R. H. et al., Scaling properties of branched polyesters, 2. Static scaling above the gel point, Macromolecules 25(1992) 7180-7187; Gorda, K. R., Peiffer, D.G., Star-shaped condensation polymers: Synthesis, characterization, and blend properties, J. Appl. Polym. Sci. 50 (1993) 1977-1983]. The above mentioned properties are very advantageous for the carrier

processing. They are preferably synthesized with ring opening [Kim, S. H. et al., Preparation of star-shaped polylactide with pentaerythritol and stannous octoate, *Macromol. Chem.* 194 (1993) 3229-3236]. Using polycondensation of polycarboxylic acids or polycarboxylic hydroxysubstituted acids with glycolic acid, the slightly cross-linked oligoesters, insoluble in water and soluble in dimethyl formamide, were prepared [Wada, R., Hyon, S.H., Ikada, Y., New biodegradable oligoesters for pharmaceutical application, *J. Biomater. Sci. Polymer Edn.* 7 (1996) 715-725]. By branching of the structure, a more convenient course of degradation, swelling and erosion can be achieved, which can positively influence the continuity of release kinetics of active compounds of the low-molecular and high-molecular types [Breitenbach, A., Li, Y. X, Kissel, T., Branched biodegradable polyesters for parenteral drug delivery systems, *J. Contr. Rel.* 64 (2000) 168-178]. Synthesis of star-shaped block copolymers of caprolactam polyols with a polylactide has been patented. Non-crystalline product with a low glass transition temperature is suitable for production of packages and for other applications (Ford, T. M., Easily degradable star-block copolymers, US Patent 5,399,666). Composition of matrices based on branched polyester carriers, prepared by very slow polycondensation of citric acid with propylene glycol, exhibits a continuous release of biological active compounds (Lindahl, A., Hagslatt, H., Bryland, R., Biologically active composition, PCTWO99/58108). Synthesis based on the copolymerisation under the ring opening of lactides and multifunctional alcohols or sugars led to formation of high molecular star-shaped copolymers suitable for preparation of bio-compatible foams (Spinu, M., Ford, T. M., Degradable foam materials, US Patent 5,210,108).

Summary of invention

The subject of the invention is a biodegradable composition with prolonged release of biologically active compounds, where the composition contains at least one biologically active compound and a carrier, which is based on the biodegradable oligoester possessing number mean molecular weight M_n from 650 to 7 500, weight mean

molecular weight M_w from 800 to 10 000 and glass transition temperature T_g from -35 to 45 °C, and which is prepared by polycondensation reaction of polyhydric alcohol containing at least 3 hydroxy groups with at least one aliphatic α -hydroxy acid in the molar ratio (polyhydric alcohol to aliphatic α -hydroxy acid) of 0.5:99.5 to 12.0:88.0, wherein the central molecule of biodegradable oligoester is a polyhydric alcohol, to the hydroxy groups of which chains created from several molecules of at least one aliphatic α -hydroxy acid are bound by ester bonds.

The composition according to the invention contains as biological active compounds preferably agents with antitumor activity, antimicrobial compounds, hormonal active compounds, immunostimulating agents, immunosuppressive agents, diagnostically useful compounds, dietary supplements or/and cosmetically useful compounds, wherein the mass ratio between the biologically active compound and the carrier is from 1:1 to 1:100 000. The composition according to the invention is preferably in the form of a homogenous one-phase solution, micellar colloid system, one-phase or two-phase gel, suspension, paste or emulsion. The composition according to the invention furthermore advantageously comprises at least one liquid biocompatible plasticizer, more advantageously soluble in the carrier and imperfectly soluble or insoluble in water, wherein the mass ratio of at least one biocompatible plasticizer to the biodegradable oligoester is from 1:20 to 9:10. The composition according to the invention further advantageously comprises at least one agent influencing the kinetics of release of the biologically active compound. The composition according to this invention further advantageously comprises at least one stabilizer of biologically active compound or a stabilizer of carrier. The process of preparation of the above mentioned composition, comprising heating of the biologically active compound, carrier, and optionally liquid biocompatible plasticizer, stabilizer of biologically active compound or stabilizer of carrier to the temperature of 35 to 75 °C and mixing them, is also a subject-matter of this invention.

The invention relates to constitution of compositions processed into the drug form of implants. The compositions are composed of two components, a carrier and a biologically active compound. The carrier

is of the oligoester type, characterised by the branched structure of the molecule formed by a central molecule of polyhydric alcohol with three or more hydroxyl groups, to which oligoester branches represented by chains of aliphatic alfa-hydroxy acids, such as lactic acid, glycolic acid, hydroxybutyric acid, hydroxyvaleric acid, hydroxycaproic acid and optionally further homologs of alfa-hydroxyacids are connected. The systems exhibit plastic viscous behaviour, fractions with lowest molecular weights play the role of plasticizers in polydisperse mixture of oligoester carrier, improving the processability and thus enabling the application. The plastic behaviour of the oligoester biodegradable carriers can be advantageously emphasized by other additives, such as liquid biocompatible plasticizers. As optional additives, release modifiers of biologically active compounds or stabilisers can be also added. These systems are preferably applied directly to the target tissue, using a syringe with a needle or other suitable devices. It is more convenient to increase the temperature of plastic systems by heating them before the application. The low level of swelling and the surface erosion of oligoester carrier are prerequisites for the surprisingly continuous and extended release of biologically active compounds with the possible consequence of enhanced biological activity.

The invention involves composition of pharmaceutical biodegradable preparations from oligoester carrier in the mixture with a biologically active compound with particular properties concerning the prolongation of release of biologically active compound. The preparation with the composition according to this procedure is intended for the parenteral application and leads to an enhanced effect of the biologically active compounds. The mixture of compounds constituting the drug form, implantable directly into the target tissue, consists of a biodegradable carrier, a biologically active compound or several such compounds, and optionally a liquid biocompatible plasticizer, and also optionally other compounds, such as release kinetics modifiers of biologically active compounds or physical stabilizers of the overall structure and texture of the

system, optionally also chemical stabilizers of individual components of the preparation according to the invention.

The biodegradable carrier is a low-molecular oligoester with a highly branched chain which is synthesized by polycondensation reaction. Multifunctional branching component is a polyhydric alcohol or sugar with three or more hydroxyl groups, such as glycerol, pentaerythritol, inositol, xylitol, mannitol, sorbitol, erythrose, threose, arabinose, ribose, gulose, idose, altrose, alose, talose, sorbose, mannose, glucose, fructose, galactose, sucrose, lactose. The chains of alfa-hydroxy acids, such as e.g. DL-lactic acid, glykolic acid, alfa-hydroxybutyric acid, alfa-hydroxyvaleric acid, alfa-hydroxy-capronic acid, are bonded by ester bonds to the molecule of polyhydric alcohol. The oligoester therefore possesses a star-shaped structure with a central molecule of polyhydric alcohol, which serves as a branching component during the polycondensation reaction. From this molecule, chains of different length are extending, while their maximum number is given by the overall number of hydroxyl groups of polyhydric alcohol or sugar.

The carrier is synthesized by polycondensation reaction of polyhydric alcohol or sugar with alfa-hydroxy acid or with a mixture of several alfa-hydroxy acids in a molar ratio of alcohol to alfa-hydroxy acids ranging from 0.5:99.5 to 12:88. The increase of the polyhydric alcohol content in the reaction mixture leads to a higher level of branching and to the limited growth of the chain. The reaction is carried out in a temperature interval from 120 to 220 °C. The rate of reaction and the maximum level of conversion achieved, expressed as a parameter of number mean molecular weight M_n , increase as the pressure decreases. The presence of a catalyst, such as for example, acidic katex, phosphoric acid or toluene sulphonic acid, increases the reaction rate and also the maximum conversion ratio achieved. The M_n value of these size polydisperse materials ranges from 650 to 7500 and the corresponding M_w value is within the interval 800-10000; as measured by the GPC method, using the calibration for linear polystyrene molecules as standards. The level of polydispersity M_w/M_n ranges from 1.2 to 12.0, usually being about 2.0. Lower-molecular fraction of oligoester carrier functions in the compositions according to the invention as a plasticizer lowering the

glass transition temperature and also the viscosity, and hence, enables better processability and applicability. The products of polycondensation reaction can be used without purification or they can be purified by precipitation from the solutions in solvents possessing the relative permittivity ϵ_r from 4.0 to 32.0, by adding some quantity of water and subsequent drying of coacervate or precipitate. It is advantageous to use nonpurified reaction products, and hence to preserve their plasticized character.

Oligoester carriers obtained by the polycondensation reaction are colourless, yellow or brown semi-crystalline or amorphous materials with their glass transition temperatures T_g ranging from $-35\text{ }^{\circ}\text{C}$ to $45\text{ }^{\circ}\text{C}$. Due to the different reactivity and the different sterical hindrance of hydroxy groups towards the esterification reaction with carboxyl, conformationally flexible oligoester chains of various length can be formed on them. This structure is consequently characterised by surprisingly strong surface activity. The random coil of these branched molecules exhibits higher density and smaller volume. As a consequence, the viscosity of their melts and their concentrated solutions is lower, compared to linear oligoesters. The melts of branched oligoester or polyester carriers, possessing the above described structure, are as biomaterials serving as drug carriers more easily processed compared to oligoesters having linear chains constitution. Another advantage in this aspect is their easier applicability in drug compositions. When satisfactorily low viscosity is achieved, it enables to apply plastic systems, formed by carriers of this type, directly to the soft tissue by a syringe or by analogous suitable devices. This results in the formation of a definite irregularly shaped bolus, which fulfils the generally known and accepted definitions of medicinal implants.

Another substantiated advantage of the branched type of oligoesters is their low swelling. Surprisingly low swelling was found in case of carriers with a high branching level and very small molecular weight. The hydrolytic degradation of the branched chain carriers has a specific course with pronounced aspects of heterogeneous type. The hydrolysis proceeds via the mechanism of a gradual diminishing of the body. Contrary to the typical heterogeneous erosion from the surface of hydrophobic material, the

erosion of oligoester particles proceeds via the mechanism of diffusion and elution of low-molecular, water-soluble degradation products into the biological hydrophilic surroundings. The body consisting of carriers of this type is in the aqueous environment or inside the organism gradually diminishing till it completely and without residues disappears. The oligoesters with linear chains, such as commercially available lactic acid/glycolic acid copolymer, degrade within the whole body, ie. homogeneously, this entirely different mechanism is given by a strong swelling of the body with continuous degradation within its whole volume, subsequent disintegration of the body into fragments and gradual dissolution and disappearance of these fragments. The hydrolysis of ester bonds in polyesters with linear chains happens randomly, no specific site with preferential cleavage within the linear chains has been found. The branched structures used in the invention have not been studied in this aspect.

The low level of swelling and the above-described heterogeneous nature of the degradation of branched carriers create conditions for continuous long-term release of biologically active compounds into the surrounding target tissues. Biodegradable materials of branched, star-shaped oligoester types are demonstrably biocompatible as carriers for biologically active compounds. They are hydrolytically cleaved by water in the biological environment to yield non-toxic compounds soluble in water, which are eliminated or metabolised.

Among compounds with anti-tumour activity, suitable for the application in the invention, it is possible to mention pharmaceuticals from the group of folic acid derivatives, such as methotrexate etc., pyrimidine analogues, such as 5-fluorouracil etc., the group of alkylating agents, such as cisplatin and derivatives thereof, complexes of platinum in oxidation state IV, the group of urea derivatives, such as carmustine (BCNU), lomustine (CCNU) etc., the group of antibiotics with an intercalation mechanism, such as antibiotics from the group of anthracycline antibiotics, such as doxorubicin, idarubicin etc., derivatives of anthracene, and anthrapyrazoles, such as mitoxantrone, oracin etc., the group of mitosis inhibitors such as vinca alkaloids, and also taxanes such as paclitaxel, docetaxel, the group of topoisomerase

inhibitors, such as topotecane, captotecine etc., etopozide, tenipozide etc., while well applicable are also compounds from the group of hormones such as gonadoliberrine analogues, gosereline, leuproline etc., group of CDK inhibitors (cycline dependant kinases), preferably trisubstituted and tetrasubstituted.

The compositions can also contain compounds influencing immunity, such as for example cyclosporine, muramyl dipeptide, muramyl tripeptide, tetanus toxoid, diphtheria toxoid, interferones, interleukines, cytokines, enterotoxines, viral and bacterial antigens, vaccines, immunogenic adjuvants for vaccination.

Among antimicrobial agents, especially suitable for compositions according to the invention are: tetracycline antibiotics, such as tetracycline and doxycycline, aminoglycoside antibiotics, such as streptomycin, neomycin, kanamycin and gentamicin, macrolide antibiotics, such as for instance erythromycin, oleandomycin, spiramycin, penicillin-derived antibiotics, such as oxacillin, amoxycillin and ampicillin, cephalosporin antibiotics, such as cephalothin, cephalazolin, cephoxitin, ansamycin antibiotics, such as rifampicin, and also lincomycin, clindamycin, spectinomycin, vancomycin and other antibiotics, such as for instance antibiotics of polypeptide type, group of cycloserine, group of chloramphenicol. Among the chemotherapeutics suitable for formulation of compositions according to the invention are: groups of sulphonamides, nitrofurans, chemotherapeutics of the type of nalidixic acid, ornidazole, among antimalarics for instance chloroquine, mepracrine, trimethoprim, among antituberculotics for instance isoniazide or rifampicin. Among other chemotherapeutics especially suitable for the formulations according to the invention are: fluoroquinolones, such as ciprofloxacin, ofloxacin, sparfloxacin, fleroxacin and others. Among compounds with antimicrobial activity the following compounds can be advantageously used: biguanide compounds, such as chlorhexidine and salts thereof, such as digluconate, diacetate, dichloride, polyhexamethylenebiguanide and so on. The quaternary ammonium salts ("quats"), such as cetrimide, benzyl dodecyl ammonium chloride, benzalconium chloride, alkylpyridinium chloride and alkylimidazolium chloride, are also suitable. Among phenolic compounds one can use according to the invention for instance

salicylic acid and esters thereof, triclosan, thymol, eugenol. Among heavy metal compounds with oligodynamic activity it is possible to mention the compounds of silver, mercury, stannum, cuprum and zinc.

With advantage, it is also possible to include to the compositions according to the invention enzymes, such as galactosidase, lysosyme and enzyme inhibitors, such as vaprotinine or orlistate.

Among compounds acting as hormones, compounds with a steroid skeleton can be used as components of compositions according to the invention. Among estrogens there are e.g. estradiol and esters thereof, ethinylestradiol, diethylstibestrol, among androgens for example testosterone and esters thereof, methyltestosterone, among androgens there are methandienone, methandriole, fluoxymestrone, nortestosteronefenylpropionate, among antiandrogens for example cyproterone-acetate, among gestagenes it is for instance northisterone and esters thereof, ethinodiolacetate, norgestrel, hydroxyprogesterone capronate, medroxyprogesterone acetate. In compositions according to the invention, it is also possible to use compounds of peptide type, proteins and glycoproteins with hormonal activity. It is also possible to use their analogues which function as agonists or antagonists of natural hormones. Among compounds with this activity there are, inter alia, leuprolid-acetate, orntid-acetate, desorelin, triptorelin, goserelin, FSH, calcitonine, somatotropins, somatostatine, vapreotide, growth hormones including recombinant hormones (BMP, EGF).

As anti-inflammatory non-steroidal compounds suitable for compositions of the given type, it is possible to mention for instance indole derivatives, such as indomethacine, arylalcanoic acid derivatives, such as ibuprofene, phenylacetic acid derivatives, such as diclophenac. It is also possible to use with advantage steroidal anti-inflammatory compounds.

Another optional ingredient of the compositions according to the invention is a liquid plasticizer, advantageously used for better application of implantable mixtures. For mixtures according to the invention, the especially preferred ones are liquid plasticizers molecularly miscible with the carrier, such as citric acid triesters, e.g. triethyl citrate, tributyl citrate, trihexyl citrate and mixture

thereof, mono- and diesters of citric and tartaric acids, lactic acid esters, such as ethyl lactate, butyl lactate, octyl lactate, dodecyl lactate, tetradecyl lactate, hexadecyl lactate and their mixtures, glycerol esters, such as triacetine, tributyrine, tricapriline, alkylesters of benzoic acid, such as C₁₀₋₁₃- or C₁₂₋₁₅-alkylesters, furthermore also dibutyl adipate, dioctylcyclohexane, octyl dekanate, cetyl stearyl octanoate etc. Plasticizers and mixture of the above mentioned and other plasticizers are characterised by molecular miscibility with oligoester carriers, however, also by a limited or very limited miscibility with water, they can even be virtually immiscible with water. They are used as minor ingredients of ternary mixtures of carrier, active compounds and optional components in concentrations ranging from 5 to 45% of weight. Liquid plasticizers as ingredients of the compositions according to the invention can be used individually or in mixtures.

As optional ingredients improving the properties of biodegradable and biocompatible implant-forming compositions according to the invention modifiers of the release of biologically active compounds can be used. These adjuvants can operate in particular in interaction with oligoester carrier by the mechanism of hydrophilisation of the mixture. Inorganic and organic salts, sugars, urea, tenzides can be used. As agents influencing the carrier degradation by changing pH in compositions according to the invention, various acids, aminoacids, amines, salts, oxides and hydroxides can be used. Biocompatible and biodegradable adjuvants in the mixtures according to the invention can influence the kinetics and the level of swelling and the rate of carrier degradation. As pharmaceutical stabilizers various compounds, used as cryoprotectants can be used, as well as hydrophilic colloids, preservatives, antimicrobial agents, antioxidants, sequestrants, osmotic agents etc.

Biodegradable implants according to this invention are prepared by mixing of weighted components, that is oligoester biodegradable carrier, biologically active compounds and optional ingredients, such as plasticizers, modifiers of pharmaceuticals release or stabilizers. The mixture is heated at a temperature ranging from 35 to 95 °C, preferably at 40 to 75 °C and more preferably at 45 to 60 °C. Thereafter it is agitated in a suitable apparatus. This highly

viscous plastic mixture can be filled in a suitable hermetically sealed vessel or to the cylinder of a syringe. The mixtures can be stored in a well sealed vessel or in a syringe of a suitable construction made from a suitable material and equipped with a suitable cap, placed in a covering protecting the mixture from air humidity and light.

The application of plastic compositions according to the invention, i.e. biodegradable carrier, biologically active compound and optional plasticizer, modifier of release or stabilizer, can be done by a syringe with tubing, trocar needle or needle of a suitable size. More viscous mixtures with higher glass transition temperatures T_g or with higher concentrations of active compounds can be heated before the application. When the composition according to the invention is applied to a tumour, it is possible to make use of the fact known from the literature dealing with physiology, concerning the temperature limit of contact compatibility. This limit is 52 °C in humans.

The advantage of the implant according to the invention is achieving better compliance in patients. The patient is less psychically and physically stressed after application of the compositions according to the invention, with several days, several weeks or several months lasting release and effect of the active compounds than in case of frequently repeated applications of biologically active compounds in a classical pharmaceutical form, such as injectable solution or suspension for injections. The release period of biologically active compound from the composition according to the invention ranges from several days to several months. This period can be influenced by the carrier type, type of structural units from reactive monomers, molecular weight of biodegradable carrier and the level of branching of the molecule. Another factor of prolongation of active compound release are physico-chemical properties of the pharmaceutical and its concentration in the implant. It has been found that the plasticizers according to this invention have surprisingly low influence on the kinetics of release of many biologically active compounds while considerably lowering the viscosity of implantable compositions. The release can be controlled by modifiers, i.e. additives influencing the osmotic, acidity and

other aspects of the systems according to this invention. The local release of often very toxic biological active compounds ensures their high concentrations in the target tissue at low levels in the circulatory system. This leads to a substantially lower incidence of undesirable side effects, i.e. toxicity, mutagenity, teratogenicity, imunogenity etc. By application of implants with compositions according to the invention with continuous long-lasting input of the biological active compound to the corresponding site, not only quantitatively, but also qualitatively novel and distinct effects in the preventive, palliative, causal or radio-sensibillised acting of biologically active compounds can be achieved.

Examples

Example 1

2000.0 mg of sorbitol/DL-lactic acid copolymer synthesised by polycondensation of both ingredients in a molar ratio 1:22, having the values $M_n = 1350$, $M_w = 2150$, $T_g = -9$ °C was mixed with 100.0 mg of paclitaxel in a beaker under aseptic conditions. The mixture was heated to 50 °C and well mixed. 200.0 mg of the resulting mixture was weighed out into vials with perforable caps. The vials were closed and sterilised by gamma-radiations with overall dose of 25 kGy.

Example 2

25.5 g of pentaerythritol/DL-lactic acid/glycolic acid terpolymer, prepared by polycondensation in the molar ratio 1:12:12 having the values $M_n = 2200$, $M_w = 4500$, $T_g = 7,5$ °C was mixed with 10.0 g of tributyl citrate and 5.5 g cisplatinum in a beaker. The mixture was heated to 70 °C and well mixed. 1.0 g aliquots of the resulting suspension were filled into syringes with 2 ml nominal volume. The syringes were closed by caps instead of needles and a plunger was set on. Everything together with a desiccant was hermetically wrapped into a foil laminated with an aluminum layer. The wrapped syringes were sterilised by the overall dose of gamma-radiations of 25 kGy and they were placed into a refrigerator.

Example 3

Irregular particles of approximately hemispherical shapes were formed by extrusion of the mixture from Example 2 at the bottom of scintillation vials. Two vials were filled by the samples weighing $m = 150$ mg, another two vials were filled by samples of $m = 450$ mg weight. The samples were overlaid with 15.0 ml of TRIS-buffer 0.05 mol.l^{-1} isotonic sodium chloride pH 6.0 and they were placed in a thermoregulated shaker with water bath at a temperature of 37 °C. The same series of sample was prepared with buffer at pH 7.0. Samples of the dissolution liquid were collected at the time intervals of 1, 3, 5, 7, 10, 14, 19, 26 and 35 days and they were analysed for the content of cisplatinum by the AAS method. After each sampling, the dissolution medium was entirely changed for a fresh one. The results of dissolution tests are stated in the following Table 1.

Table 1

m (mg)	pH	X	1	3	5	7	10	14	19	26	35
150	6.0	X ₁	11.6	21.5	32.7	47.2	62.8	87.9	96.9	104.2	104.7
		X ₂	14.3	23.0	35.6	50.4	64.9	91.5	99.4	101.6	103.1
	7.0	X ₁	16.7	24.8	35.2	48.6	64.8	84.1	93.2	94.0	96.6
		X ₂	17.7	28.4	39.3	54.9	64.0	87.5	96.8	100.5	100.9
450	6.0	X ₁	0.9	8.7	23.8	43.6	52.7	68.1	83.5	93.2	95.6
		X ₂	6.5	11.3	27.0	37.2	47.9	63.8	84.8	94.9	95.9
	7.0	X ₁	5.4	12.6	31.7	43.8	58.4	70.3	75.8	83.0	89.6
		X ₂	6.2	11.2	31.4	47.2	62.6	82.7	83.3	94.2	96.3

Based on the results stated in Table 1, it can be concluded that the release of cisplatinum is not dependent on the actual acidity of the system. The rate of release of the cisplatinum decreases with the increase of the implant size.

Example 4

Metothrexate (2.50 g) was mixed with 2.50 g poloxamer 407 and 20.0 g DL-lactic acid/glykolic acid/mannitol terpolymer to yield the sample 4A. The terpolymer was synthesized in molar ratios of the reactants 5: 47.5:47.5 and had the values $M_n = 2650$, $M_w = 5800$ and $T_g = 19.1$.

Binary mixtures containing 10% of metothrexate and 90% of terpolymer (sample 4B), and further ternary mixture of 10% metothrexate, 30% tributyrine and 60% terpolymer (sample 4C) and also quaternary mixture of 10% metothrexate, 30% tributyrine, 0.5% zinc stearate and 59.5% terpolymer (sample 4D) were prepared analogously.

The mixtures were heated at 70 °C and mixed in a ultrasonic field of Ikasonic 50 U probe. The mixtures were filled at 2.00g +/- 0.10 g aliquots into syringes. The syringes were set on by a plunger and wrapped into a foil laminated with an aluminum layer. Wrapped syringes were sterilised by the overall dose of gamma-radiations 25 kGy and stored in refrigerator at 3 °C.

Example 5

150 mg of implantable mixtures from Example 4 were placed to the bottom of scintillation vials. Two vials with weighed mixtures from each of the experiments using 4A, 4B, 4C and 4D mixtures were prepared. The mixtures were overlaid with isotonic phosphate-citrate buffer at pH 7.0 and the vials were tightly closed by screwing cap. Then they were placed in a shaking thermostat tempered at 37 °C. The samples were collected at the intervals of 1 day, 3 days, 7 days, 14 days, 21 days and 28 days, and measured on spectrophotometer at $\lambda = 283$ nm. The results of liberation tests are stated in the following Table 2, demonstrating release of metothrexate from the matrices according to Examples 4 and 5.

Table 2

Time (days)	4A	4B	4C	4D
1	14,6	2,5	2,1	2,9
	25,7	2,9	3;0	1,5
3	30,5	7,4	13,8	7,6
	39,8	8,2	13,5	5,3
7	45,3	12,4	18,4	11,8
	53,2	10,6	27,4	12,0
14	57,9	40,7	55,3	33,7
	62,1	38,2	50,1	45,8
21	63,0	51,4	60,4	67,6
	64,6	58,3	66,7	68,8
28	65,7	74,6	70,9	101,1
	65,9	77,0	76,2	95,3

It is clear from the results of Table 2 that the release of metothrexate is long-term and continuous. The system is flexible. The required rate of liberation can be controlled by suitable additive and by its optimised concentration.

Example 6

This example demonstrates the growth of mouse plasmacytoma ADJ/PC6 in inbred mice BALB/c. The anti-tumour effect of biodegradable injectable composition according to Example 2 was tested by its intra-tumoral administration to inbred mice BALB/c.

In this experiments, inbred mouse of the strain BALB/c, females, their body mass ranging from 10 to 20 g, were used, and they were s.c. transplanted a mouse plasmacytoma of the line ADJ/PC6 to the right hip. When tumours achieved the size of approximately 1 cm³, a single dose applied 0.1 ml of the formulation preheated at 45 to 50 °C, containing 0.0 mg CDDP/kg and 16.0 mg CDDP/kg or 35.5 mg CDDP/kg was applied intratumorally.

The animals were divided into four groups, each comprising six animals: 1. tumours with no application, i.e. the control group, 2. group with tumours and i.t. applied carrier alone, i.e. placebo group, 3. i.t. applied 16.0 mg of CDDP/kg, 4. i.t. applied 35.5 mg of CDDP/kg.

During the experiments the tumours were measured and the weights of mice were determined (every 3. or 4. days) and the tumour volumes were calculated using the equation $l \times w \times \pi/6$. The day 35 after the transplantation, i.e. day 20 after application of the composition, the mice were put to death, the tumours were weighed and the % tumour growth inhibition (%TGI) and tumour weight inhibition (%TWI) were calculated. $\%TGI = (1 - (\text{average volume of tumour in treated group} / \text{average volume of tumour in control group})) \times 100$; $\%TWI = (1 - (\text{average weight of tumour in treated group} / \text{average weight of control group})) \times 100$. The tumour growth inhibition (percentage) and tumour weight inhibition (percentage) on day 35 after the start of experiment are stated in the following Table 3.

Table 3

Group	CDDP dose	%TGI	%TWI
1. Control	-	0	0
2. Placebo	-	5.16	4.46
3.	16.0 mg / kg	99.81	98.66
4.	35.5 mg / kg	99.84	99.84